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Influence of sodium pretreatments on the preparation of Ag-doped polycationic bentonite for antibacterial purposes

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Abstract. Sodium bentonites have excellent cation exchange capacity (CEC) giving them great power of silver adsorption, which enables their use as precursors of antimicrobial materials. Countries like Brazil, however, do not have natural sources of sodium bentonite, only calcium or polycationic clays that do not show the same adsorption and ion exchange potential. In this study, the adsorption of silver in a polycationic bentonite from Quatro Barras, Brazil, previously subjected to a sodium treatment is evaluated. Samples were first modified with Na_2CO_3 or NaOH and then, subjected to silver impregnation in a batch system under controlled ambient conditions. Antibacterial properties of silver-exchanged clays were evaluated by the disk susceptibility and the minimum inhibitory concentration tests on *Escherichia coli* and *Staphylococcus aureus* bacteria. Results show that the sodium treatment with Na_2CO_3 allowed higher concentrations within a shorter time, increasing the pH without compromising the montmorillonite structure, which resulted on greater CEC and swelling values. Such better performance of the samples previously treated with sodium carbonate also caused an enhanced silver adsorption, resulting on a material of greater antibacterial potential. Experimental adsorption data fitted well to Freundlich isotherm.

Keywords. Bentonite; silver; antibacterial activity; sodium treatment; ion exchange.

1. Introduction

The development of inorganic antibacterial materials have always attracted great interest due to several advantages over traditionally used organic agents, like chemical stability, thermal resistance, safety, long lasting action period, simple manufacturing and effectiveness against a broad spectrum of bacteria and fungi [1,2]. They usually occur in the form of composites with metallic ions, like Ag^+, Zn^{2+} and Cu^{2+}, which possess the natural ability to inhibit microbial growth.

Ag-based antibacterial materials have been widely studied, and despite some disadvantages, such as high cost and reactivity, a bactericidal action that can be derived from very diverse mechanisms and interactions with different cell elements, and which is still not fully understood [3–5], silver is still the most used for this purpose: it is very effective against bacteria, it acts under very low concentrations, it is relatively inert and tend to be safe in respect with human health. It has been demonstrated that silver ions play a key role in bactericidal action of silver-based materials, being formed by simple and inexpensive synthesis, presenting strong interaction with ceramic matrices, therefore, being slow and minimally released in aqueous media, and showing efficiency in inhibiting several microorganisms [3,4,6]. Wakshlak *et al* [7] also reported that silver ion-killed bacteria also present a capability of killing living bacteria, creating a residual effect and prolonging the bactericidal action of the Ag^+ source material.

Montmorillonite is the main clay mineral of bentonite, and has a 2:1 lamellar structure formed by two silica tetrahedral sheets sandwiching an alumina octahedral sheet, composing a crystal lattice separated by an interlayer space containing water and exchangeable cations (Na or Ca), whose proportion gives them a classification into sodium or calcium montmorillonites, or even polycationic ones, when the interlamellar cations are equivalent to each other. The classification of the montmorillonite as sodium, calcium and polycationic can be extended to the bentonites that they compose.

Sodium bentonites are good carriers of metallic ions because of their higher cation exchange capacity (CEC) and adsorption, ion selectivity, surface area and negative superficial charge [8]. Besides, they are economic, viable, chemical inert and non-toxic [1,9]. However, natural sodium bentonites occur profusely in the Western United States with smaller deposits in only nine other countries, like Argentina, Canada and South Africa [10]. Elsewhere, including Brazil, calcium or polycationic bentonites are much more common, but present inferior properties compared with sodium clays.

Previous studies report the sorption and/or antimicrobial effects performed with bentonite [1,11–15]. However, these studies focus on the use of natural clays or other treatments (thermal, mechanic, or the production of composites). So, there is an essential gap in the studies related to this subject and also a significant economic limitation of a majority of countries that do not possess natural reserves of sodium bentonites, which endorses the relevance of the present paper.

The main objective of this work was to study the influence of a previous treatment for polycationic bentonite with sodium compounds (NaOH and Na_2CO_3) for the Ag^+ ion exchange and subsequent antibacterial activity. This paper reports the preparation, characterization and antibacterial potential of polycationic bentonite submitted to sodium pretreatment, followed by ion exchange process with Ag ions. The antibacterial activity was evaluated through growth inhibition of *Escherichia coli* and *Staphylococcus aureus*, measured by the disk susceptibility test. The minimum inhibitory concentration (MIC) was determined by broth microdilution.

2. Materials and methods

2.1 *Materials and chemicals*

Raw clay samples were from city of Quatro Barras, Brazil (25°22′2″ S, 49°4′30″ W). This region contains the largest Brazilian bentonite reserve (45.6% of total), but it is the least commercially explored, corresponding only to 4.6% of the total national production [16]. The raw clay consists of a low-grade bentonite, namely, clay with great amounts of impurities and gangue minerals, despite a major presence of clay minerals of finer particle size, especially polycationic montmorillonite. All clay samples were previously subjected to mechanical processes of attrition (by mechanical stirring at 1500 rpm for 60 min of 50% solid dispersions) followed by wet sieving at 200 mesh to eliminate the coarse particles and reduce the fraction of undesirable accessory minerals.

All chemicals used in the experiment were of analytical grade: sodium hydroxide (NaOH, VETEC, 99.5%) and sodium carbonate (Na_2CO_3, VETEC, >99%) were used to prepare the solutions applied in sodium pretreatments. Silver nitrate ($AgNO_3$, Sigma-Aldrich, >99%) was used to prepare the silver solutions applied in the equilibrium studies.

2.2 *Material characterization*

X-ray diffraction (XRD) analysis was conducted using the powder method with a PW 1830 Phillips X-ray diffractometer using CuKα (λ = 1.5418 Å) as incident radiation, operating at 40 kV and 30 mA with step size of 0.02°.

Local chemical analysis by energy dispersive X-ray spectrometry (EDX) was performed by an EDX spectroscope attached to a XL 30 Phillips scanning electron microscope.

Swelling index was determined by the standard Foster technique, by measuring the amount of gel formed, 24 h after the slow addition of 1 g of clay in 100 ml of distilled water.

CEC was determined by adsorption of methylene blue, based on the standard test method for methylene blue index of clay [17]. In this procedure, CEC values were calculated from the methylene blue volume added to the clay dispersion, when the saturation point could be visually observed through the formation of a halo around the mixture drop placed on a filter paper.

2.3 *Sodium pretreatments*

The samples of raw clay were submitted to a surface treatment aiming at the ion exchange between Ca^{2+} interlayer ions from montmorillonite and Na^+ ions from the reagent solution, to improve the sodium interlayer content.

Pretreatment with sodium compounds was carried out by placing the raw polycationic bentonite (B/Raw), in a ratio of 100 g l^{-1}, in contact with solutions of 0.472 M Na_2CO_3 or 0.25 M NaOH, under constant stirring (IKA RW 20, 450–700 rpm) and temperature (25.0 ± 1.5°C). The stirring time was 24 h for the Na_2CO_3 and 72 h for the NaOH solutions. Solution pH was measured before and after sodium treatment through a pH meter (Lucademo, LUCA-210). After the contact time, the samples were washed with distilled water to remove excess reagent from the surface and then with deionized water, vacuum filtered and oven dried at 65°C. The sodium content in each sample was identified by CEC analysis. The operating conditions for each of the pretreatments were defined in preliminary statistical analysis [18]. The samples submitted to CEC treatment with Na_2CO_3 and NaOH were named B/Na_2CO_3 and B/NaOH, respectively.

2.4 *Equilibrium studies*

Ag^+ sorption behaviour was investigated, in batch system and in duplicate, adding 1 g of the samples to 60 ml of $AgNO_3$ solution with concentration range of 1–30 mmol l^{-1}, under constant agitation (150 rpm) and at 30°C in a Dubnoff shaker water bath (Ethik, 304-TPA) with protection from light exposure. The pH of the samples was measured at the beginning and at the end of the experiment. Sorption assays were conducted at controlled room temperature because it has been shown that silver sorption is favourable at relatively low temperatures [12], and also to evaluate the formation of antibacterial material at simpler and lower cost conditions.

After 2 h of contact, samples were collected, centrifuged (Quimis, Q222T) and the residual silver present in the supernatant was analysed by atomic absorption spectroscopy (Shimadzu AA-7000). The ultraviolet–visible (UV–Vis) spectra of the clay before and after impregnation with silver were also measured on a Shimadzu UV-18000 UV–Vis spectrophotometer operated at a resolution of 1 nm.

2.5 *Antibacterial activity*

Antibacterial activity was evaluated through growth inhibition of *S. aureus* (ATCC 6538) and *E. coli* (ATCC 25922), measured by the disk susceptibility test (Kirby-Bauer) and the MIC by broth microdilution.

The silver exchanged samples from natural and sodium-activated bentonites were prepared as follows: a 0.575 M solution of $AgNO_3$ (corresponding to the CEC of the raw clay) was added to the clay dispersions and maintained with a continuous stirring for 2 h. Then, all the samples were washed with deionized water by vacuum microfiltration, until no Ag^+ was verified in the passing solution (AgCl test). Precautions were taken for the light sensitivity of silver.

For the disk susceptibility technique, a preincubated bacterial culture was inoculated and grown in Petri dishes containing plate count agar with a Ni–Cr stem, to form a 'carpet' of bacteria. The agar was then pierced ($d = 10$ mm) and the holes were filled by aliquots of 100 mg of clay sample and 80 ml of distilled water to moisten the clay and thus provide a better diffusion. The width of inhibition given by diffusion halos was measured and photographed after 24 h of incubation at 37°C.

MIC was determined by broth microdilution. Portions of 10 mg of each sample were dispersed in 1 ml of 10% DMSO and, subsequently, serial dilutions were prepared from these to concentrations up to $1000\,\mu g\,ml^{-1}$. Volumes of 10 ml of each dispersion were distributed in holes of a microdilution plate, to which 85 ml of Müeller–Hinton agar was added. Then, 5 ml of bacterial inoculums was added, corresponding to a 10^7 CFU ml^{-1} dispersion of *E. coli* and *S. aureus* and the plates were incubated for 24 h at 37°C. The readings of the experiments were performed using a microplate reader (CLX800-Biotek model). A revealing solution of the bacterial growth [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium-chloride) was used, where the turbidity of bentonite interfered in the observation.

3. Results and discussion

3.1 *Material characterization*

The XRD pattern of the natural clay (figure 1) revealed the presence of montmorillonite, $Na_{0.3}Al_2(Si, Al)_4O_{10}(OH)_2\cdot 2H_2O$, with a (001) interlayer spacing of 15.63 Å (5.65°, 2θ). Appreciable amount of quartz and kaolinite and minor
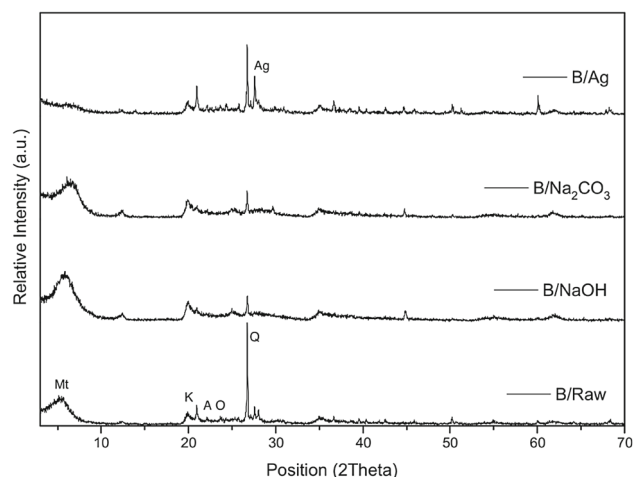
Figure 1. XRD patterns of the montmorillonite samples: raw (B/Raw); treated with sodium hydroxide (B/NaOH) and sodium carbonate (B/Na$_2$CO$_3$); and modified with silver (B/Ag).

quantities of albite and orthoclase were also observed, denoting the heterogeneous nature of the raw clay.

The diffractograms of samples B-NaOH and B-Na$_2$CO$_3$ showed shifts towards right of the (001) reflection, which can be interpreted as the result of ion exchange and modification of the hydration state of the interlayer cations [19]. The observed removal and/or decrease of the non-clay minerals were probably due to dissolutions in alkaline medium.

The sample modified with silver presented the broadening and disappearance of the basal (001) reflection, indicating that the silver intercalation produced total exfoliation and collapse of the layered montmorillonite structure and consequent smaller crystallite sizes. A small additional reflection at 28° (2θ) corresponding to the (111) Ag reflection, points out the existence of silver in the structure of the clay mineral and, hence, a successful interchange of interlayer sodium cations.

Elemental chemical analysis by EDX was carried out in all the samples and the obtained data are summarized in table 1. After sodium activation, there was a decrease in the mass percentage of Ca^{2+} and a direct increase in the percentage of Na^+, indicating a positive occurrence of ion exchange, especially for the sample activated with Na$_2$CO$_3$. The same occurred to the silver-treated samples, where all Ag^+ detected, represented a diminution of the interlayer sodium, which can be associated with a preferential exchange for this ion, as reported by Magaña *et al* [1].

The properties of CEC and swelling are presented in figure 2. Commercial sodium bentonites usually present values of CEC ranging between 80 and 150 mEq per 100 g of clay and a swelling index $>8\,ml\,g^{-1}$. However, the raw clay used in this work showed insignificant values for both properties (swelling index around $2\,ml\,g^{-1}$ and CEC of 42.5 mEq per 100 g). This result can be attributed to a heterogeneous mineralogical constitution and a poor content of interlayer sodium.

Table 1. Elemental quantification of raw bentonite (B/Raw), bentonite treated with sodium hydroxide (B/NaOH) and sodium carbonate (B/Na$_2$CO$_3$); and bentonite doped with silver (B/Ag, B/NaOH/Ag, B/Na$_2$CO$_3$/Ag).

	Mass composition, %					
	Before impregnation with Ag			After impregnation with Ag		
Element	B/Raw	B/NaOH	B/Na$_2$CO$_3$	B/Raw/Ag	B/NaOH/Ag	B/Na$_2$CO$_3$/Ag
Si	59.7	40.7	39.3	34.4	34.6	35.2
Al	17.6	15.3	16.0	15.4	15.2	15.1
Fe	6.9	12.7	13.8	11.3	11.5	11.2
Ca	0.3	ND	ND	ND	0.8	0.4
K	2.8	1.4	1.4	1.5	1.5	1.3
Na	1.1	1.9	3.0	ND	0.9	2.1
Mg	1.5	2.2	2.3	2.2	2.4	2.4
Ti	0.7	2.9	2.6	1.2	1.0	1.0
Ag	ND	ND	ND	1.7	2.5	3.3
O	8.1	22.0	21.5	32.3	29.5	28.0
C	1.3	0.9	ND	ND	ND	ND

ND, not detected.
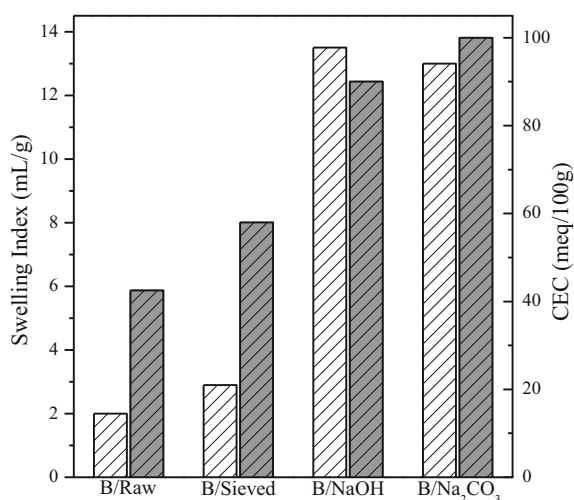
Figure 2. Properties of the clay samples subjected to different treatments: white column, swelling index; gray column, CEC.

Sample preparation by wet sieving partially increased the value of both the properties (swelling index = 3 ml g^{-1} and CEC = 58 mEq per 100 g). Vigorous mechanical stirring de-agglomerates the clay components, leaving the coarser particles (impurities) free from the clay minerals (finer) and thus, they end up being separated at screening. The CEC value after sieving is only related to the montmorillonite, which is the component that most accomplishes the ion exchange. It is also possible to verify that the treatments with sodium significantly increased the value obtained for the studied properties. The CEC value obtained for B/Na$_2$CO$_3$ sample is around 95 mEq per 100 g, which is very close to that obtained by Mosser-Ruck and Cathelineau [19] for pure homoionic Na-bentonites from

Wyoming, USA (CEC = 101 mEq per 100 g); and swelling index was just 1.4 times smaller than the ones obtained by Alther [20] for Na$^+$-rich raw bentonites from Wyoming (around 18 ml g^{-1}).

According to Ijagbemi *et al* [21], montmorillonite has two types of electric charges: a variable charge (pH-dependent) resulting from the proton adsorption/desorption reaction of the sites present on the surface, and a structural negative charge resulting from the isomorphic substitution in the clay mineral structure. This statement indicates that the ion sorption can be described as involving a coordination reaction at specific sites of the surface and by an electrostatic interaction between the ions and the charged surface. In this sense, when comparing the pH values of NaOH (B/NaOH pH = 8.8) and Na$_2$CO$_3$ (B/Na$_2$CO$_3$ pH = 10.5) solutions at optimal concentrations with the initial pH solution with the raw clay (B/Raw = 3.5), it can be concluded that the results obtained for sample B/NaOH are slightly lower than those obtained for B/Na$_2$CO$_3$ due to the role of the pH solution in the electrostatic attraction process, since for Na$_2$CO$_3$ solution, which has higher pH, the material surface tends to have a higher negative surface charge, which favours the fixation of the cations and, consequently, the increase in the CEC (more information can be found in ref. [18]).

Although the pH plays a significant role on sodium activation, it is important to highlight that the pH during activation was not different for NaOH and Na$_2$CO$_3$ (see figure S1 in supplementary data file). Both solutions presented similar pH variations related to the reagent concentration, but the treatment with Na$_2$CO$_3$ at higher concentrations and pH resulted on greater CEC and swelling values, which did not happen to the treatment with NaOH (where the CEC and swelling both got reduced by the increasing concentration and pH). So, the diverse effects of OH$^-$ and CO$_3^{2-}$ on the structure of

montmorillonites could explain the differences between each reagent. At higher concentrations, the significant amount of hydroxide ions lead to the dissolution of the primary silicate from clay minerals, as showed in previous studies [22,23]. On the other hand, Hayashi and Yamada [24] showed that montmorillonites subjected to alkaline environments based on carbonate ions first display predominance on the cation exchanges, before the dissolution of silicates and aluminates from the clay mineral structure. This also explains why a reduced time of activation (24 h) for the treatment with Na_2CO_3 resulted in a better performance—at longer periods of agitation, the disruption of the clay mineral begins to take place, overlapping the cation exchange process, as already mentioned in previous works [18].

3.2 *Silver impregnation by ion-exchange process*

The equilibrium curves for silver sorption by the raw polycationic bentonite and by samples submitted to different pretreatments with sodium showed that the treatment with Na_2CO_3 presented the best adhesion of silver ions, followed by treatment with NaOH and the raw material (figure 3).

According to Giraldo *et al* [25], montmorillonites have some atoms in their tetrahedral structure, such as Si^{4+}, Mg^{2+} and Al^{3+}, which can be replaced by other atoms of similar atomic size (isomorphic substitutions) and due to this, negative charges are found in all the layers. These negative charges leading to the clay mineral with the ability to absorb positive charges within the interspace, attracted by electrostatic forces, such as light ions Na^+, K^+ and Ca^{2+}. In addition, the higher the sodium content in the interlayer space of a montmorillonite, the greater the adsorption capacity of silver by the clay mineral due to the fact that sodium has a greater power of hydration and lower valence, which allows the adsorption of several water molecules, thereby reducing the bond strength along the clay mineral surface, separating the lamellar layers and making the Na^+ ions more susceptible of being replaced. Furthermore, the monovalent cations of sodium and silver display ionic isomorphism, which facilitates the interchange between them. This mechanism is also observed by increasing the CEC of the material with sodium pretreatment, since the concentration of Na^+ ions, which are more easily exchangeable, is higher in these samples.

With respect to the concentration of silver in solution, the number of ions sorbed per unit mass of bentonite samples (q_{eq}) increases gradually with increasing Ag^+ concentration, until reaching an equilibrium at $\sim$25 mg Ag per litre, obtaining a q_{eq} of 27.8 mg g^{-1} for B/Raw, 69.9 mg g^{-1} for B/NaOH and 111.9 mg g^{-1} for B/Na$_2$CO$_3$.

The UV–Vis spectra of the best precursor material (sodium exchanged bentonite by Na_2CO_3 treatment) before and after impregnation with silver presented important differences Na_2CO_3 (see figure S2 in supplementary data file). The presence of a broad absorption peak at about 260 nm in both the curves is assigned to the formation of bonds between sodium and silver cations with oxygen [18], as observed with Ag^+ exchanged zeolites [26]. The reduction in the intensity of absorption for the silver-treated bentonite is a reflection of the difference in absorptivity between Ag–O and Na–O bonds, which indicates the successful intercalation of silver. The absence of an optical plasmon resonance absorption peak at about 305 and 425 nm, which are respectively, attributed to Ag aggregates and metallic nanoparticles of Ag [27,28], shows that silver maintained an ionic nature.

Toward a better understanding of the silver sorption behaviour onto clays samples, Freundlich [29], Temkin [30], and Dubinin and Radushkevich [31] equilibrium models were used to adjust experimental data (figure 4 and table 2).

The Freundlich model presented R^2 values >0.93 for all the samples tested, suggesting that the model is applicable for all the clays tested. For the Temkin model, the R^2 value was only >0.9 for the B/Na$_2$CO$_3$ sample, whereas for the Dubinin–Radushkevich model, $R^2 > 0.9$ was obtained for the B/Raw sample and for B/Na$_2$CO$_3$. The performance of these equilibrium models was evaluated using the F-test [32], comparing the reduced chi-square values (S_R^2) obtained for each clay, and indicated that the Freundlich model was statistically more adequate to describe the equilibrium data of silver adsorption for all bentonite samples. Similar results were obtained in other studies [11,13,15].

The suitability of the Freundlich model to the experimental data suggests a multilayer adsorption of the silver ions. In addition, the values obtained for the parameter n are >1 for all the samples, indicating that the affinity between sorbent and sorbate is relatively strong [33] and the silver adsorption process is favourable ($0.1 < 1/n < 1$) [34]. Moreover, for the statistical theory of adsorption when the ratio $1/n < 1$,
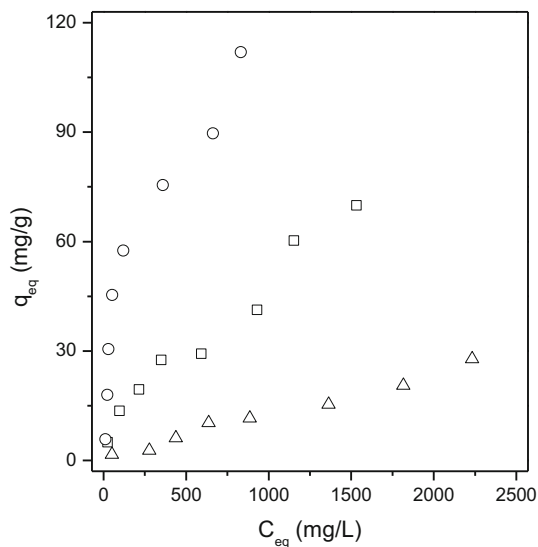
Figure 3. Silver sorption onto clays subjected to different treatments: $\triangle$, raw montmorillonite; $\square$, montmorillonite sodium activated with NaOH; $\bigcirc$, montmorillonite sodium activated with Na_2CO_3.
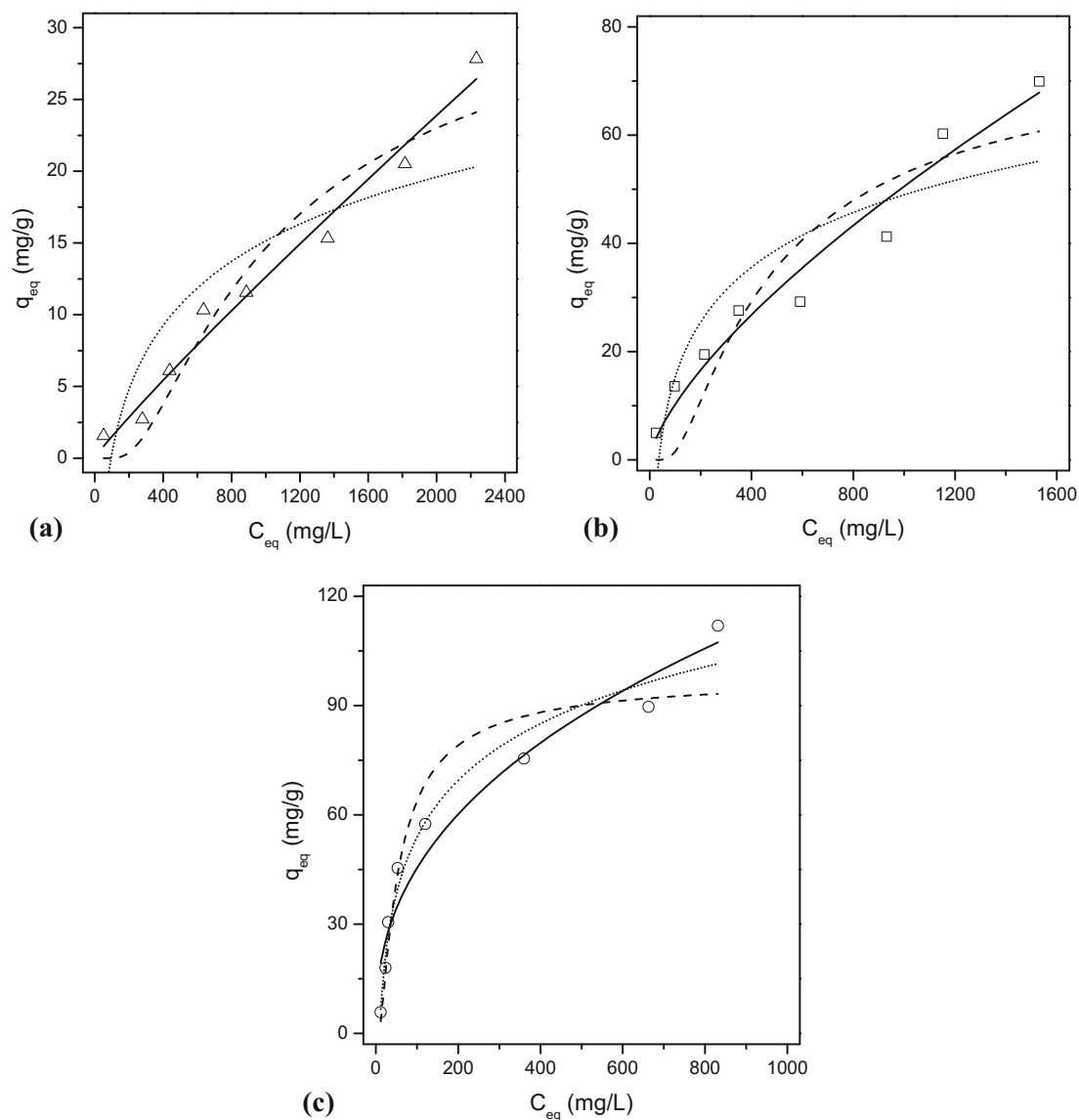
Figure 4. Equilibrium isotherms for the silver adsorption onto: (**a**) raw montmorillonite; (**b**) montmorillonite sodium activated with NaOH and (**c**) montmorillonite sodium activated with Na_2CO_3: continuous line, Freundlich model; dotted line, Temkin model; dashed line, Dubinin–Radushkevich model.

the material presents heterogeneous surfaces with minimum interactions between the adsorbed ions [35]. The values for this ratio for sodium-treated bentonite samples are farther from 1 and confirm the high heterogeneity of the adsorbent and that the sorption was more favourable in these materials. This is also reinforced by an expressive increase of k_F, especially for the sample treated with Na_2CO_3, confirming a higher adsorption capacity for the sodium-treated clays, following the same increasing trend: B/Raw < B/NaOH < B/Na_2CO_3.

Therefore, it is possible to prove that the adsorption of silver occurred through weak van der Waals interactions, associated with relatively low-bonding energies. For antimicrobial purposes, that could be beneficial, since the silver ions must be easily desorbed from the ceramic matrix to make contact with the microorganisms that they will inhibit.

3.3 *Antibacterial activity*

Results of the disk susceptibility test are showed in figure 5. The inhibition zone diameters and diffusion lengths from the edge of each sample in the agar plate are given in mm for *S. aureus* (figure 5a) and *E. coli* (figure 5b). The test was repeated thrice, and the results correspond to the mean values. Raw and sodium-activated samples (with no silver) exhibited no antimicrobial potential.

Table 2. Equilibrium parameters from Freundlich, Temkin and Dubinin–Radushkevich models for the adsorption of silver onto clays subjected to different treatments.

| Model	Parameter	Treatment		
		B/Raw	B/NaOH	B/Na$_2$CO$_3$
Freundlich	n	1.1 ± 0.1	1.4 ± 0.2	2.5 ± 0.3
	k_F (mg$^{1-1/n}$l$^{1/n}$ g^{-1})	0.02 ± 0.01	0.4 ± 0.2	7 ± 2
	R^2	0.976	0.956	0.936
	S_R^2 (mg^2 l^{-2})	2.0	22.2	85.7
Temkin	b_T (J mol^{-1})	386 ± 89	170 ± 34	110 ± 8
	k_T (l g^{-1})	0.011 ± 0.005	0.03 ± 0.01	0.11 ± 0.02
	R^2	0.722	0.779	0.967
	S_R^2 (mg^2 l^{-2})	22.5	112.4	44.0
Dubinin–Radushkevich	k_{DR} (mol^2 J^{-2})	36 ± 5	79 ± 13	98 ± 7
	B_{DR} (J mol^{-1})	0.36 ± 0.07	0.16 ± 0.05	0.018 ± 0.003
	R^2	0.922	0.818	0.918
	S_R^2 (mg^2 l^{-2})	6.3	92.5	109.8
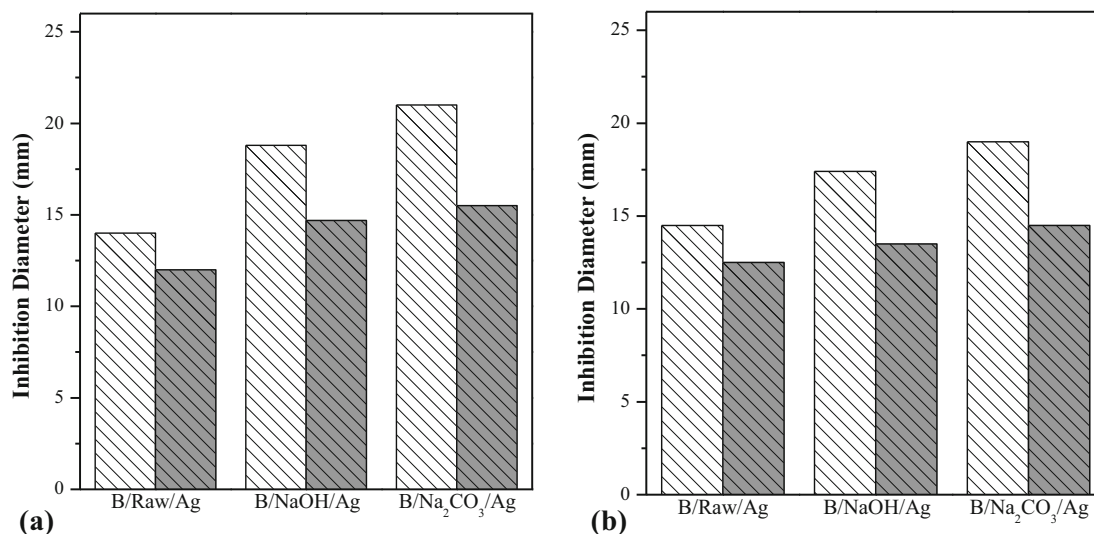
Figure 5. Inhibition zone diameters and diffusion lengths for (**a**) *S. aureus* and (**b**) *E. coli*. White column, diffusion halo; gray column, total diameter.

Raw bentonite treated with silver showed results similar to those presented in the literature with inhibition diffusions varying from 2.0 to 3.0 mm, whereas alkaline activated samples, later modified with silver, showed very significant increases in the halos diameter.

The activity of the samples disposed as dispersions were slightly smaller than the ones obtained by the powdered samples, which can be attributed to the high instability of silver ions in aqueous solutions, possibly leading to the formation of metallic silver of lower antibacterial action. The presence of Ag^0 in aqueous samples could be visually verified in the disk susceptibility test itself, where a thin coating of metallic aspect was formed over the sample, in contrast with the dry samples that did not exhibit the same appearance (figure 6).

Results of the MIC are presented in table 3. MIC value means the lowest concentration of sample able to inhibit bacterial growth. The raw clay, even after modification with silver, caused no inhibition at any concentration. The Ag-bentonite, as opposed to it, showed satisfactory inhibition concentrations—specially the one pretreated with Na$_2$CO$_3$—with improved values in respect with some other studies [1,36].

Application of pure AgNO$_3$ for antimicrobial purposes has limited utility for several reasons, such as salt interfering effects, discontinuous release of inadequate concentrations of Ag ions [37] and solubility of Ag ions in Cl$^-$ containing biological and environmental media, since AgCl has very low solubility and precipitates rapidly from solution [38].

(a)

(b)
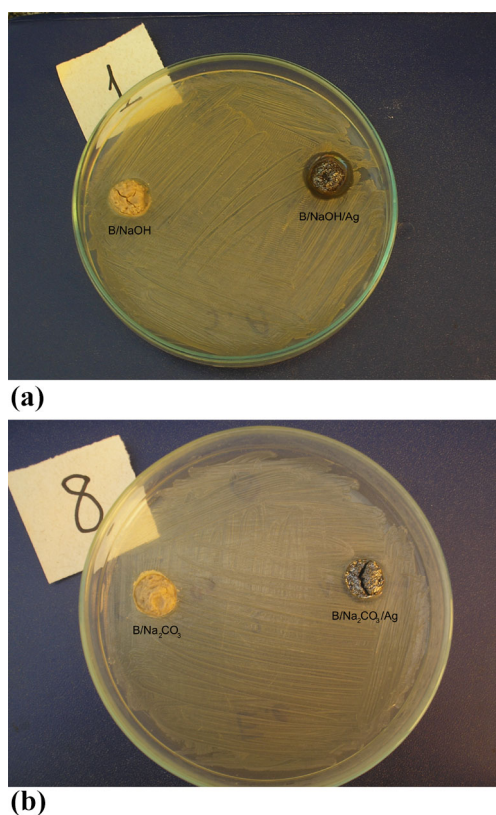
Figure 6. Occurrence of Ag^0 coating of the moisten samples: (**a**) *S. aureus* and (**b**) *E. coli*.

Table 3. MIC results of the clays subjected to different treatments, over *S. aureus* and *E. coli* bacteria.

	MIC values, $mg\,l^{-1}$	
Sample	*S. aureus*	*E. coli*
B/Raw	N.I.	N.I.
B/NaOH	N.I.	N.I.
B/Na$_2$CO$_3$	N.I.	N.I.
B/Raw/Ag	N.I.	N.I.
B/NaOH/Ag	416.67	1000.00
B/Na$_2$CO$_3$/Ag	250.00	500.00
AgNO$_3$	500.00[1]	1000.00[1]

N.I., no inhibition; [1]Baran [39].

Regarding the free Ag ions control test, Baran [39] used AgNO$_3$ with brand and purity identical to those used in this study and obtained a MIC of 0.5 mg ml^{-1} for *S. aureus* and 1.0 mg ml^{-1} for *E. coli*, demonstrating that the bentonite composite treated with Na$_2$CO$_3$ and silver had a twice better results than the reagent alone (MIC = 250 mg l^{-1}, or 0.25 mg ml^{-1} for *S. aureus* and 500 mg l^{-1}, or 0.5 mg ml^{-1} for *E. coli*). Importantly, the lower the MIC value, the greater the efficiency of antimicrobial activity, as it corresponds to the lower concentration capable of totally inhibiting bacterial growth. For bentonite treated with NaOH and silver, the results obtained in this study (MIC = 417 mg l^{-1} for *S. aureus* and 1000 mg l^{-1} for *E. coli*) were not significantly different from those presented by the free Ag control test presented by Baran [39]. Another study developed by Lima and co-workers [40] presents the control test using free Ag ions for the inhibition halo, obtaining the value of 12 mm. Comparing this result with those presented in figure 5, it is observed that the halos obtained using all bentonite composites were superior to the control test presented by Lima and co-workers [40], which confirms the good performance of the material.

4. Conclusion

Bentonite impregnated with silver inhibited growth of *S. aureus* and *E. coli* bacteria. Pretreatments with NaOH and Na$_2$CO$_3$ showed better antibacterial activities, which was the result of a satisfactory sodium interlayer exchange on montmorillonites enabling a subsequent stronger adsorption of Ag$^+$ ions. Freundlich model presented the best adjustment and confirms the high heterogeneity of montmorillonites with respect to silver adsorption. Values of equilibrium constants suggest that sodium treatments of clays, especially the one with Na$_2$CO$_3$, provide them with higher adsorption capacity and surface heterogeneity.

Clays beneficiated with sodium carbonate presented the best overall results, what can be associated with a higher pH (hence, a more negative surface), without collapsing the structure of the clay minerals that compose them. By this mean, pretreatments with Na$_2$CO$_3$ can be considered as a good option for bentonites with insufficient sodium interlayer for the development of antibacterial clays.

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